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Optimization of Crystallization Process Condition of Nutmeg Seed Oleoresin

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Abstract. Nutmeg (*Myristica fragrans* Houtt) is a native spice from Indonesia. It contains oleoresin which has a unique aroma. Extraction of nutmeg seed oleoresin is carried out by ultrasound assisted extraction (UAE). The shelf life of nutmeg seed oleoresin can be extended by microencapsulation which is conducted by crystallization using sucrose as coating material. This study was aimed to optimize the crystallization condition process of nutmeg seed oleoresin. The formulation used were nutmeg seed oleoresin (5 gram, 10 gram, 15 gram), sucrose (30 gram, 35 gram, 40 gram), and water (15 gram, 20 gram, 25 gram). Physicochemical characteristics measured were moisture content, water activity (a_w), color, total oil, surface oil, trapped oil, and antioxidant activity. Optimization of process conditions were analyzed by Response Surface Method. The results showed that nutmeg seed oleoresin (13.266 gram), sucrose (35.047 gram) and water (20.019 gram) produced the optimum antioxidant activity of microencapsulated oleoresin. Furthermore, the application of nutmeg seed oleoresin (11.088 gram), sucrose (36.381 gram) and water (19.331 gram) resulted in the optimum trapped oil percentage of microencapsulated oleoresin. These methods can be applied to produce nutmeg seed oleoresin powder which then could be used as spices and beverage ingredient.

Keywords: crystallization, nutmeg seed, oleoresin.

1 Introduction

Indonesia is one of the largest spices producers in the world. Generally, the produced spices in Indonesia are exported in the whole form. However, during distribution, loss of quality often happens because of insects and microorganisms that certainly can make negative impact to spices producers. Spices take an important role to give taste and aroma on food processing product. Besides being used as seasoning, spices also widely used for medicine, soft drinks ingredient, and main material for cosmetics. The use of whole form spices has some disadvantages such as varied strength and quality in taste and aroma, possible contamination by microorganisms, unstable during storage, and more amounts that needed in usage. Therefore, spices processing product development is needed.

One method to process spices is by extracting the spices using organic solvent to get oleoresin. Oleoresin is a compound that obtained from spices extraction using organic

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solvent. Oleoresin is spices extract that has complete flavor characters. It contains flavor main components which are volatile compound (essential oil) and non-volatile compound (resin and gum) that each of them takes a role in determine aroma and taste [2]. One of extraction methods that potential to be applied is *ultrasound-assisted extraction* (UAE). UAE method is chosen because it has efficient result, simple, use low temperature, less amount of solvent and energy [3-4].

To ease the usage and handling, oleoresin can be microencapsulated and processed into dried powder. Microencapsulation is a method of coating liquid or solid form of core ingredients using specified coating material that makes core particles have desired physic and chemical properties. Microencapsulation can protect the ingredient from evaporation, oxidation, and chemical reaction [5].

One of the microencapsulation methods is crystallization. Crystallization is formation of solid particles in homogenous phase that have reached super saturation condition. Super saturation is condition where solid (solute) concentration in solution exceeds the solution saturated concentration. Super saturation condition can be improved through four methods: changes of temperature, solvent evaporation, chemical reaction, and solvent composition [6]. Microencapsulation of nutmeg seed oleoresin can be conducted by crystallization method using sucrose as a coating material. The objective of this research is to optimize the crystallization condition process of nutmeg seed oleoresin.

2 Materials and Methods

2.1.1 Materials

Materials used in this research consist of Nutmeg Seed (*Myristica fragrans* Houtt), ethanol 96%, methanol 99.98%, aquadest, sucrose and DPPH (2,2-diphenyl-1-picrylhydrazyl). Whilst the tools used in this research consist of ultrasonic cleaner UC-10SD, rotary vacuum evaporator, digital thermometer, chromameter, a_w meter, centrifuge, spectrophotometer, moisture analyzer, analytical balance, blender, saucepan, paddle, stove, Erlenmeyer, oven, filter paper, filter paper Whatman no. 1, vial tubes and sieve (36 and 80 mesh size).

2.1.2 Methods

This study used an experimental design with Response Surface Methodology which obtained 17 treatments, 3 of them being the center point treatment.

2.1.2.1 Extraction of Nutmeg Seed Oleoresin

Dried nutmeg seeds are grinded into powder. Then the nutmeg seed powder was dissolved with ethanol 96%, the ratio of solid to solvent was 1:10. Extraction was started by soaking Erlenmeyer that contained sample in the ultrasonic cleaner UC-10SD water bath for 37 minutes at 50°C using frequency at 45 kHz and power 100. Then sample was taken and filtered out with filter paper Whatman no. 1. The obtained filtrate was separated from solvent using rotary vacuum equipment.

2.1.2.2 Microencapsulation of Nutmeg Seed Oleoresin

Saucepan and paddle were prepared. Oleoresin was weighed for 5, 10 and 15 grams. Sucrose was weighed for 30, 35 and 40 grams. Water was weighed for 15, 20 and 25

grams. Then sucrose, water, and thermometer were put into saucepan. The fire was turned on at the lowest level. Sucrose solution was stirred until it reaches a temperature of 120°C. Then, oleoresin was put into saucepan. Sucrose solution and sample were stirred until it reaches a temperature of 120°C. Stove was turned off while stirring quickly until crystal is formed. Crystallization yield was taken and grinded with blender to get the crystallized powder.

2.1.2.3 Color Analysis

Color testing was done with chromameter. Firstly, chromameter was calibrated by putting chromameter on a white plate. Then, tested sample was put into clear plastic and ensured not be tangled. Testing result appeared on the screen as L*, a*, and b* value. If L* value or lightness is positive, then sample has light color, while negative indicates dark color. The value of a* shows tendency to red (+) and green (-), while b* value shows tendency to yellow (+) and blue (-) color [7].

2.1.2.4 Water Content Analysis

A clean empty aluminium pan was put into moisture analyzer and ensured pan was in the right position. Then, the cover was closed. The equipment will automatically perform tare by pressing the tare button. Sample was weighed for 0.5 gram and leveled up on the pan. The sample was heated until it reached a constant water content (around 10 minutes).

2.1.2.5 Water Activity (a_w) Analysis

Water activity analysis (a_w) was done using a_w meter. Firstly, container for sample was ensured in clean and dry conditions before putting the sample in until the height reached half of the container's height (± 15 grams). The analysis was done for 15 minutes.

2.1.2.6 Total Oil (TO) Analysis

TO analysis was done by weighing microencapsulate sample for 2 grams. Then sample was poured into an Erlenmeyer. Ultrasonic cleaner UC-10SD was turned on and filled with aquadest. The equipment is set up at 50°C, 15 minutes, and frequency 45 kHz. The obtained filtrate was moved into porcelain bowl which was known the weight before and put into oven for 24 hours. Then, the porcelain bowl that contained sample was put into desiccator for 15 minutes. The bowl was weighed as final weight. The result was calculated using the formula: TO (gram) = final weight (gram)-initial weight of bowl (gram) (modification of [8].

2.1.2.7 Surface Oil (SO) Analysis

SO analysis was started by weighing the sample for 2 grams. Then, the sample was put into centrifuge tube and added 10 ml of ethanol. The tube was put into centrifuge equipment and set at 1700 rpm for 15 minutes. Then, sample was filtered using filter paper and washed with 15 ml ethanol twice. The obtained filtrate was moved to porcelain bowl which was known the weight before and put into oven for 24 hours. The sample was put into desiccator for 15 minutes. The bowl was weighed as final weight. The result was calculated using the formula: SO (gram) = final weight (gram)-initial weight of bowl (gram) (modification of [9-10].

2.1.2.8 Trapped Oil Analysis

The result of trapped oil is obtained from calculation using formula: trapped oil (gram) = (total oil (TO) (gram)-surface oil (SO)(gram))/(initial sample weight)×100% (modification of [11].

2.1.2.9 Antioxidant Activity Analysis

The analysis was started by weighing microencapsulate for 0.125 gram. Then sample was dissolved with 5 ml ethanol and rested it for 2 hours until the residue is sedimented. The supernatant was taken for 0.1 ml and dissolved with 3.9 ml DPPH solution. The sample was stored for 30 minutes, wrapped up with aluminium foil in the dark room. After it, analysis was done using spectrophotometer at 517 nm. The control was made by adding 0.1 ml ethanol and 3.9 ml DPPH solution and rested it for 30 minutes.
Antioxidant activity is calculated using formula:

Antioxidant activity (%) = [(control absorbance-sample absorbance)/(control absorbance)]× 100 (modification of [9].

3 Data Analysis

Data analysis was conducted using Statistica 6.0 Response Surface Methodology (RSM). Sample treatment randomization was based on Central Composite Design (CCD) system using 3 levels which are -1, 0 and +1. Independent variable (x) consists of oleoresin concentration (5, 10 and 15 grams), sucrose (30, 35 and 40 grams), and water (15, 20 and 25 grams). Response (Y) consists of analysis of water content, water activity (a_w), color, antioxidant activity and trapped oil. Center point (0) was repeated for 3 times in 2 batch, so resulting 17 runs in each batch. The runs come from 2k+2k+no formula, where 'k' was the amount of independent variable and 'no' indicates the amount of repetition in every analysis [12]. Analysis was continued with analysis of variance to find out the independent variable that affects to the response significantly, fitted surface to find out 3-dimensions surface graphic of response to independent variable, Pareto chart to find out the effect of independent variable which either positive or negative to the response, critical values to find out independent variable of optimum response, and regression analysis to find out prediction of response equation mathematically. Regression application was done through prediction of mathematic response equation [13].

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{12}x_1^2 + b_{22}x_2^2 + b_{32}x_3^2 \qquad (1)$$

Note:

y	: response of water content, water activity (a_w), color, antioxidant activity and trapped oil
b	: offset term (constant model) of water content, water activity (a_w), color, antioxidant activity and trapped oil
b_1, b_2, b_3	: linear effect coefficient of oleoresin, sucrose and water
b_{12}, b_{13}, b_{23}	: interaction effect coefficient of oleoresin and sucrose, oleoresin and water sucrose and water
b_{12}, b_{22}, b_{32}	: quadratic effect coefficient of oleoresin, sucrose and water

4 Results and Discussion

Physicochemical characteristics measured (moisture content, water activity, color, total oil, surface oil, trapped oil, and antioxidant activity) were shown at Table 1 and 2. Optimization of process conditions were analyzed by Response Surface Method.

Table 1. a_w, antioxidant activity, total oil, surface oil and trapped oil of microencapsulated nutmeg seed oleoresin analyzed by RSM.

No	Oleoresin (g)	Sucrose (g)	Water (g)	Moisture Content (%)	a_w	Antioxidant Activity (%)	Total Oil (%)	Surface Oil (%)	Trapped Oil (%)
1	5.00	30.00	15.00	3.58±0.35	0.80±0.01	54.74±29.49	54.00±1.27	20.25±1.91	33.75±0.64
2	5.00	30.00	25.00	3.49±0.13	0.78±0.02	77.43±12.83	57.15±1.77	20.25±2.62	36.90±4.38
3	5.00	40.00	15.00	2.73±0.56	0.78±0.01	85.80±4.25	50.80±0.49	22.10±0.00	28.70±5.09
4	5.00	40.00	25.00	3.15±0.93	0.79±0.02	70.44±13.83	56.15±0.49	21.50±3.54	34.65±3.04
5	15.00	30.00	15.00	2.51±0.37	0.57±0.01	89.59±1.69	51.50±5.23	24.08±7.95	27.43±2.72
6	15.00	30.00	25.00	3.06±0.69	0.74±0.01	92.56±1.91	59.90±14.14	28.23±8.45	31.68±5.69
7	15.00	40.00	15.00	2.26±0.24	0.72±0.03	92.45±2.28	66.55±6.01	26.05±3.32	40.50±9.33
8	15.00	40.00	25.00	2.99±0.12	0.75±0.05	91.42±0.12	64.20±7.70	31.30±0.14	32.90±6.93
9	1.59	35.00	20.00	2.93±5.63	0.80±0.01	44.39±0,88	52.45±6.01	17.68±0.81	34.78±5.20
10	18.41	35.00	20.00	2.57±4.04	0.72±0.06	93.02±2.25	67.80±14.28	29.10±3.11	38.70±11.17
11	10.00	26.59	20.00	3.20±0.09	0.73±0.04	92.91±2.70	53.30±2.40	30.88±1,17	22.43±3.57
12	10.00	43.41	20.00	2.97±0.32	0.78±0.00	92.61±2.14	64.50±2.26	27.45±3.32	37.05±1.06
13	10.00	35.00	11.59	3.39±0.54	0.76±0.00	92.85±1.90	63.60±6.51	25.05±8.70	38.55±2.19
14	10.00	35.00	28.41	3.54±0.34	0.73±0.05	91.34±4.46	60.60±6.22	27.53±9.44	33.08±3.22
15	10.00	35.00	20.00	3.67±0.92	0.69±0.12	92.06±1.82	66.80±3.54	22.15±6.43	44.65±2.90
16	10.00	35.00	20.00	3.44±0.33	0.72±0.04	92.84±2.04	68.80±4.67	22.60±7.07	46.20±2.40
17	10.00	35.00	20.00	3.55±0.46	0.73±0.04	92.64±1.95	67.55±0.07	22.50±6.08	45.05±6.15

Table 2. Color intensity of microencapsulated nutmeg seed oleoresin analyzed by RSM.

No	Oleoresin (g)	Sucrose (g)	Water (g)	Color intensity		
				L*	a*	b*
1	5.00	30.00	15.00	83.13±4.7	2.70±3.00	15.32±3.99
2	5.00	30.00	25.00	79.25±1.27	4.94±0.07	17.84±0.98
3	5.00	40.00	15.00	57.75±34.3	3.73±0.82	17.08±0.94
4	5.00	40.00	25.00	56.37±30.6	4.41±0.03	16.51±1.19
5	15.00	30.00	15.00	72.86±2.18	8.04±0.42	19.55±0.39
6	15.00	30.00	25.00	48.82±21.8	8.67±0.00	21.21±1.25
7	15.00	40.00	15.00	52.55±26.2	7.88±0.49	20.81±0.81
8	15.00	40.00	25.00	51.96±28.7	7.65±0.56	21.61±0.74
9	1.59	35.00	20.00	60.18±37.6	1.60±0.74	12.45±1.40
10	18.41	35.00	20.00	49.63±27.1	9.09±0.12	21.55±1.85
11	10.00	26.59	20.00	67.89±4.27	7.26±1.09	20.24±0.12
12	10.00	43.41	20.00	71.12±4.21	6.33±0.85	20.33±0.24
13	10.00	35.00	11.59	66.35±8.27	7.28±0.08	20.40±0.08
14	10.00	35.00	28.41	72.58±2.77	7.19±0.04	20.74±2.94
15	10.00	35.00	20.00	74.78±1.88	6.73±1.43	19.10±2.81
16	10.00	35.00	20.00	72.32±2.45	6.95±0.41	20.26±0.97
17	10.00	35.00	20.00	72.00±1.81	7.26±0.33	20.50±1.64

4.1 Water Content

Previous research mentioned that more addition of nutmeg seed oleoresin generated the lower water content even though not significant. It is because of solid content enhancement from extract in the end of water content analysis [14]. This hypothesis is supported by Pareto Chart (Fig. 1) that addition of oleoresin treatment linearly affected to water content negatively but not significant. Negative effect means more addition of oleoresin, water content will be getting lower. The obtained data is appropriate with the previous research.
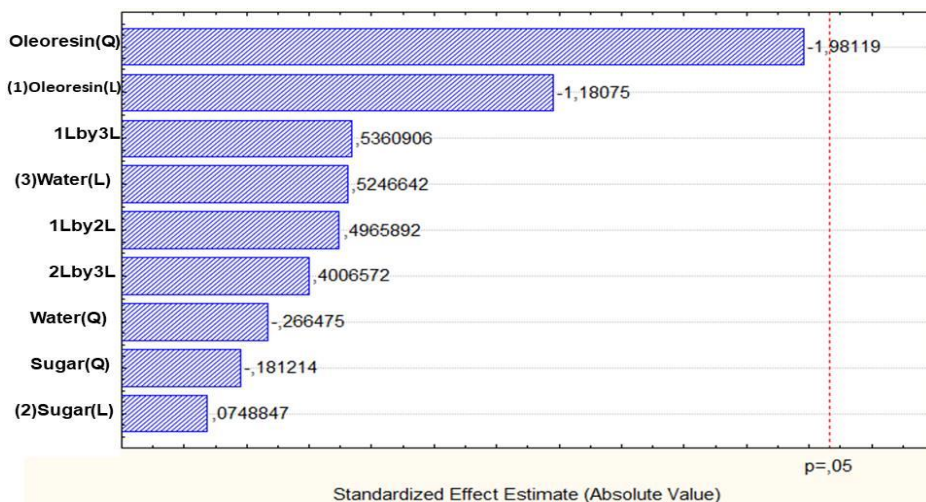

Fig. 1. Pareto Chart of Water Content Standardized Effects

4.2 Water Activity (a$_w$)

Water activity (a$_w$) is defined as total free water. Free water supports the growth of microbes, chemical and enzymatic reaction, and food damage. The initial hypothesis states that more addition of nutmeg seed oleoresin, water activity will be getting lower significantly [14]. Oleoresin contains volatile compounds (essential oil) and non-volatile compounds (resin and gum) [2] that is not counted as water. Therefore, it does not increase the amount of water especially free water. This hypothesis is supported by the Pareto Chart (Fig. 2). It shows that linearly addition of oleoresin is the most significant treatment which generates in the negative effect on the water activity. A negative effect means that the more oleoresin is added, the lower the water activity.
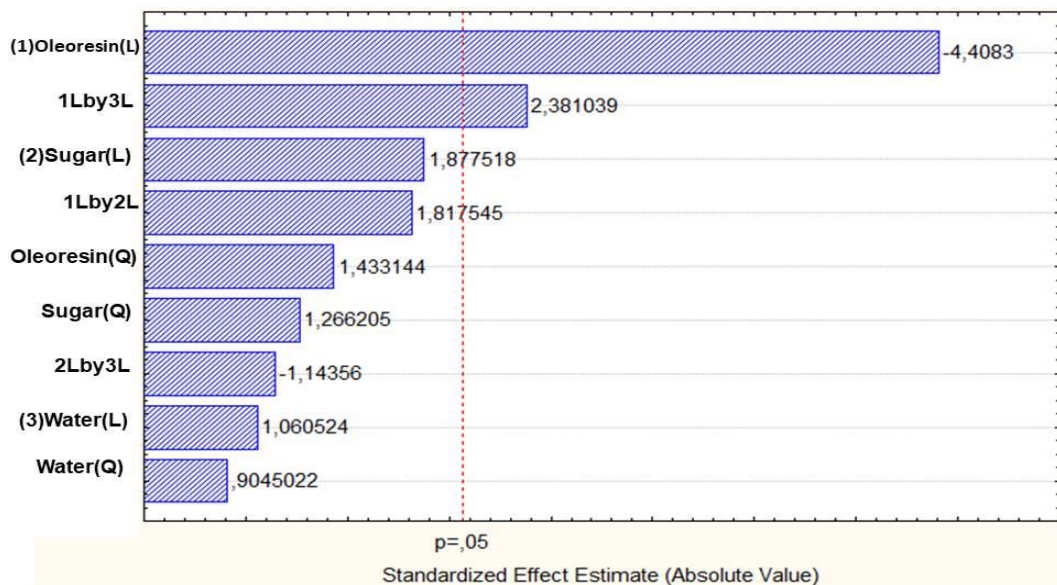

Fig. 2. Pareto Chart of a$_w$ Standardized Effects

4.3 Color Analysis

Based on [7], L* value shows the lightness of sample. L* value is positive reveals that sample has light color, while negative value shows dark color. The previous study mentioned that more addition of marjoram leaves extract generated lower L* value even though not significant [14]. They also said that L* value gets lower as the concentration of the extract increases. Based on Table 1., samples had L* values in the range between 49.63 and 83.13 (Table 2), it means samples had a quite light color.

Based on [7], a* value shows tendency to red (+) and green (-) color. The addition of oleoresin linearly was the treatment that most takes an effect to enhancement of a* value. Based on Table 1., samples had a* value in the range between 1.60 and 8.67 (Table 2), it means samples were tended to have red color. The value of b* shows tendency to yellow (+) and blue (-) color. Linear addition of oleoresin was the treatment that most takes an effect to enhancement of b* value. Based on Table 1., samples had b* value in the range between 12.45 and 21.61 (Table 2), it means samples were categorized in yellowish color.

4.4 Antioxidant Activity

According to Maya et al [14], nutmeg extract contains myristicin, elemicin, safrole, and sabinene. These compounds contribute to the level of antioxidant activity which provides health benefits. The initial study stated that addition of more extract resulted in significantly higher antioxidant activity [15]. Table 3 shows that linear addition of oleoresin was the most takes an effect treatment significantly and positively to antioxidant activity. Affect positively means oleoresin addition causes enhancement antioxidant activity value. The result is supported by the initial study conducted by [15].
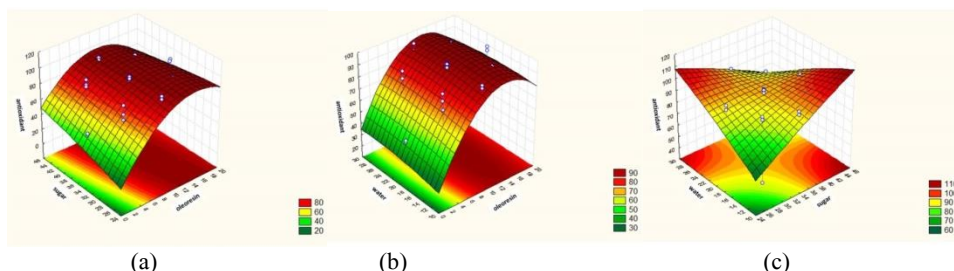

| (a) | (b) | (c) |

Fig. 3. Fitted response surface of (a) oleoresin and sucrose addition effect to antioxidant activity (b) oleoresin and water addition effect to antioxidant activity (c) sucrose and water addition effect to antioxidant activity

Based on the regression coefficient table (Table 3), the equation of response function for antioxidant activity is

$$y = -203.172 + 13.913x_1 + 6.833x_2 + 8.755x_3 - 0.112x_1x_2 - 0.027x_1x_3 - 0.210x_2x_3 - 0.357x_1^2 - 0.016x_2^2 - 0.026x_3^2 \quad (2)$$

On all three antioxidant activity fitted response surfaces, antioxidant activity of the samples generally lied in the range between 44.39% and 93.02%. Critical value of antioxidant activity (Table 4), shows that predicted value of antioxidant activity was achieved at 96.477% with the application of oleoresin 13.27 grams, sucrose 35.05 grams and water 20.02 grams.

Table 3. Regression coefficient values of the quadratic polynomial model

Factor	Regression coefficient value	
	Trapped Oil	Antioxidant Activity
Mean	-313.921	-203.172
(1)Oleoresin (L)	-0.020	13.913*
Oleoresin (Q)	-0.122*	-0.357*
(2)Sucrose (L)	15.758*	6.833
Sucrose (Q)	-0.221*	-0.016
(3)Water (L)	7.559	8.755
Water (Q)	-0.135*	-0.026
1L by 2L	0.108	-0.112
1L by 3L	-0.062	-0.027
2L by 3L	-0.045	-0.210*
R^2	0.633	0.773

*significant

Table 4. Critical Values of Antioxidant Activity

Factor	Observed Minimum	Critical Values	Observed Maximum
Oleoresin (gram)	1.591	13.266	18.409
Sucrose (gram)	26.591	35.047	43.409
Water (gram)	11.591	20.019	28.409
Predicted value		96.477	

4.5 Trapped Oil Analysis

The previous research mentioned that addition of more water resulted in lower trapped oil because of longer evaporation time and crystal formation [6]. Longer evaporation time is caused by higher water content; therefore longer time is needed to get super saturation condition. The longer super saturation condition causes the longer crystal formation. The previous study also stated that addition of more sucrose resulted in higher trapped oil. More coating material can coat ingredients effectively and protect them from heat damage [1].

Table 2 shows that addition of sucrose has a linear, significant and positive effect on the trapped oil. Linear addition of oleoresin and water is not significant to trapped oil. Positive effect means that addition of sucrose causes enhancement of trapped oil value.
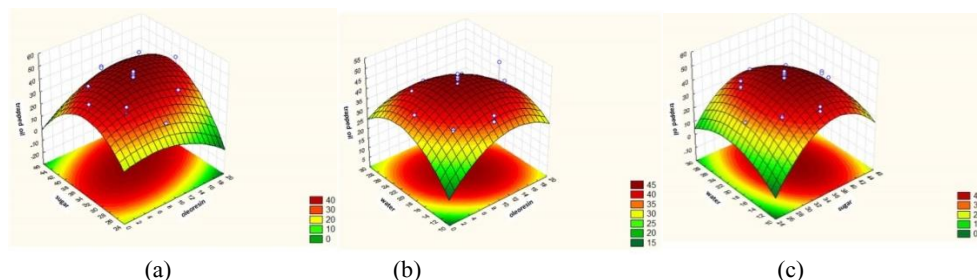

(a)	(b)	(c)

Fig. 4. Fitted response surface of (a) oleoresin and sucrose addition effect to trapped oil (b) oleoresin and water addition effect to trapped oil (c) sucrose and water addition effect to trapped oil.

On all three trapped oil fitted responses surface, trapped oil of the samples generally lied in the range between 22.43% and 46.20%. Critical Values in Table 4 shows that the predicted value of trapped oil will be achieved at 45.684% with the application of oleoresin 11.09 grams, sucrose 36.38 grams and water 19.33 grams. Based on Table 2., the equation of response function for trapped oil was

$$y = -313.921 - 0.020x_1 + 15.758x_2 + 7.559x_3 + 0.108x_1x_2 - 0.062x_1x_3 - 0.045x_2x_3 - 0.122x_1^2 - 0.221x_2^2 - 0.135x_3^2 \qquad (3)$$

Trapped oil is the main parameter because it can show process efficiency of crystallization [11].

Table 2. Critical Values Result of Trapped Oil

Factor	Observed Minimum	Critical Values	Observed Maximum
Oleoresin (gram)	1.591	11.088	18.409
Sucrose (gram)	26.591	36.381	43.409
Water (gram)	11.591	19.331	28.409
Predicted value		45.684	

5 Conclusion

The linearly addition of oleoresin generated a positive and significant effect to a* value, b* value and antioxidant activity, but significantly showed a negative effect on a_w and not significant to water content and lightness. The linearly addition of sucrose significantly generated a positive effect to the trapped oil. The linearly addition of water showed no significant effect to the physicochemical characteristic. Optimum antioxidant activity (96.477%) analyzed by RSM was obtained using the formula of oleoresin 13.266 grams, sucrose 35.047 grams and water 20.019 grams. Whilst the optimum trapped oil (45.684%) was obtained using the formula of oleoresin 11.088 grams, sucrose 36.381 grams and water 19.331 grams.

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